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TRANSITIONS BETWEEN HELICAL AND PROTOFIBRILLAR CONFIGURATIONS IN DOUBLET AND SINGLET MICROTUBULES IN SPERMATOZOA OF *STYLOCHUS ZEBRA* (TURBELLARIA, POLYCLADIDA)[1, 2]

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Microtubules occur widely in both plant and animal cells. They may exist either as singlets or doublets. The arrangement of microtubules within the cell is often precise, as exemplified by the 9 + 2 pattern of doublet and singlet microtubules in cilia, flagella and axial units (Fawcett and Porter, 1954), the double spiral of singlet microtubules in the axopodia of Heliozoa (Tilney and Porter, 1965), the parallel rows in the axostyle of certain flagellates (Grimstone and Cleveland, 1965), or the single rows of singlets which parallel the long axis of asymmetric cells (Byers and Porter, 1964) and nuclei (Burgos and Fawcett, 1955). Whether or not the component microtubules of these widely differing associations are functionally and/or structurally equivalent is not known. Similarities in size, appearance, and staining properties have been cited to suggest that cytoplasmic singlet microtubules and ciliary microtubules may be similar, and that these, in turn, are similar to spindle microtubules (for references, see Behnke and Forer, 1967). Turner (1968) has also concluded that centriolar triplets are identical with other types of microtubules described.

Biochemical studies tend to support this view, at least in part. The actin-like, nucleotide-containing protein described from the flagella of starfish spermatozoa (Plowman and Nelson, 1962) has been found to occur in the outer fibers of the flagella of sea urchin spermatozoa and in the cilia of *Tetrahymena*, and has been shown to contain a guanine nucleotide (Stephens, Renaud and Gibbons, 1967). The protein of the outer fibers has a molecular weight of *ca.* 59,000 in the flagella of sea urchin spermatozoa (Stephens, 1968) and 55,000 in cilia of *Tetrahymena* (Renaud, Rowe and Gibbons, 1968). Shelanski and Taylor (1967, 1968) have found a similar protein with a molecular weight of 60,000 in the central and outer fibers of sea urchin sperm flagella, and have demonstrated a guanine nucleotide binding site in both components of the flagella and a colchicine binding site in the central elements. The fact that a colchicine binding site has not been demonstrated by these workers in doublets has been attributed to the preparative methods used (Shelanski and Taylor, 1968).

The mitotic spindle apparatus has also been found to be composed of a protein similar to actin by Roslansky (Mazia, 1955), with a molecular weight of 66,000 ±

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7000 (Kane, 1967) and with a colchicine binding site (Borisy and Taylor, 1967). It would appear, then, that ciliary doublet and singlet microtubules and mitotic spindle microtubules are composed of similar subunits. No attempt has been made to analyze the composition of cytoplasmic microtubules, other than those constituting the spindle.

In spite of the similarities that have been shown to exist among microtubules, there is increasing evidence that all are not identical. Based on the effects of such physical and chemical agents as cold, heat, colchicine, and pepsin on the microtubules of cranefly and rat spermatozoa, and rat tracheal cilia, Behnke and Forer (1967) have described four classes of microtubules: (1) A-tubules of ciliary doublets; (2) B-tubules of ciliary doublets; (3) central and accessory tubules of cilia; and (4) cytoplasmic tubules and spindle tubules. This classification of the A- and B-tubules is in agreement with the demonstration that ATPase, in the form of dynein, is contained in the "arms" which are associated only with the A-tubule (Gibbons, 1963, 1965, 1966; Gibbons and Rowe, 1965). Also, differences in solubility have been shown to exist between the A- and B-tubules of *Chlamydomonas reinhardii* (Jacobs, Hopkins and Randall, 1968). The A- and B-tubules of the lungfluke spermatozoon are affected differently by treatment with pepsin (Burton, 1968), and the two subtubules stain differently with ethanolic phosphotungstic acid following fixation with osmium tetroxide (Gordon and Bensch, 1968). Furthermore, cytoplasmic singlets and spindle microtubules in many forms are susceptible to cold treatment and colchicine (Tilney and Porter, 1967; Tilney, 1968; Inoué and Sato, 1967), whereas ciliary microtubules are not (Burton, 1968).

Numerous studies suggest, however, that the four classes of microtubules described by Behnke and Forer may have to be further subdivided. Jacobs *et al.* (1968) have reported that the two central singlet ciliary microtubules of *Chlamydomonas* do not have identical solubilities. Allen (1968) has demonstrated in sectioned material the presence of arms on only one of the central tubules in cilia of *Tetrahymena,* and Chasey (1969) has found projections from only one of the central tubules in negatively stained preparations from the same material.

Furthermore, all cytoplasmic microtubules may not be identical. Behnke and Zelander (1967) have subdivided cytoplasmic microtubules into O-tubules (those preserved by osmium fixation) and G-tubules (those preserved by glutaraldehyde fixation, but not by osmium fixation) and have shown that G-tubules are more sensitive to certain solutions at a neutral pH than are O-tubules.

Burton (1966b) has found that the cortical singlet microtubules in spermatozoa of the lungfluke have a helical configuration, whereas cortical microtubules of a trypanosome show no evidence of this helical pattern. The spermatozoa of the lungfluke are unaffected by treatment with cold or colchicine, in this way resembling ciliary microtubules more than cytoplasmic ones (Burton, 1968). In addition, by use of the Markham rotation technique, cytoplasmic singlets of the lungfluke sperm have been estimated to be made up of 8 subunits per turn of the helix (Burton, 1966a), whereas 12 or 13 have been reported, using the same technique, in meristematic cells of *Juniper* (Ledbetter and Porter, 1964), in *Chlamydomonas* (Ringo, 1967), and in spermatozoa of *Sciara* (Phillips, 1966).

Perhaps the difference between the points of view that microtubules are identical and that they differ can be resolved by showing that some, if not all, micro-

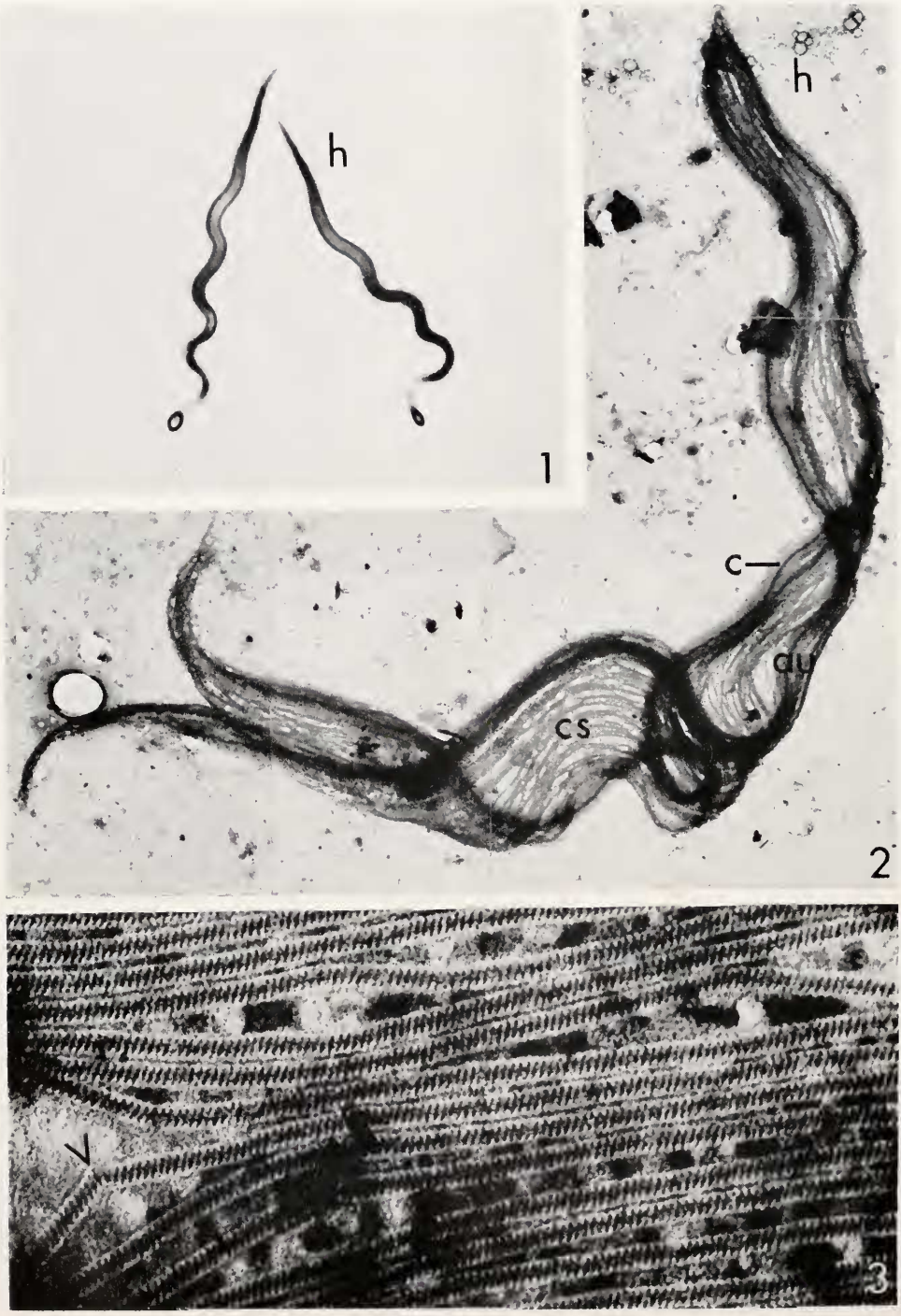
tubules are composed of similar subunits, and that the differences in the effects of various treatments result from differences in their macromolecular associations. That polymorphism can result from slight variations in the associations of similar subunits has been discussed by Casper (Satir, 1970). As a possible example, doublets and some singlets have been shown to exhibit both helical and filamentous configurations in negatively stained material. Cortical singlets with a helical configuration in lungfluke spermatozoa have been observed to undergo a transition from the helical to a filamentous configuration, in which approximately 5 beaded, longitudinal protofibrils occurred in negatively stained preparations (Burton, 1966b). Behnke and Zelander (1967) have observed protofibrils in microtubules of mammalian blood platelets. Protofibrils have been reported frequently for doublet microtubules negatively stained with phosphotungstic acid (Pease, 1963; André and Thiéry, 1963; Grimstone and Klug, 1966). Grimstone and Klug (1966) have shown by optical diffraction that intact doublets display definite longitudinal periodicities, and they interpret their results in terms of a helical surface lattice. A helical configuration in negatively stained doublet microtubules in spermatozoa of *Dugesia* is shown in a micrograph by Silveira (Porter, 1966). Pease (1963) has shown that the subfibers of doublets, when separated from one another, assume a helical configuration. André and Thiéry (1963) have demonstrated the presence of a helical pattern in the A-tubule of human spermatozoa. There is evidence, therefore, that doublets and some cytoplasmic singlets treated with phosphotungstic acid may exist in either a protofibrillar or a helical form.

In the present study, axonemal doublet and cortical singlet microtubules of the spermatozoa of the polyclad, *Stylochus zebra*, were negatively stained with phosphotungstic acid (PTA) (Brenner and Horne, 1959). The helical and protofibrillar configurations of these two classes of microtubules were compared in an attempt to ascertain whether or not the same subunits can exist in both configurations, in both types of microtubules.

Materials and Methods

Specimens of *Stylochus zebra*, obtained from the Supply Department of the Marine Biological Laboratory at Woods Hole, Massachusetts, were maintained in dishes of sea water in the laboratory at room temperature. Spermatozoa were removed from the animals by transecting the vas deferens with needles and applying pressure along the ducts to free the spermatozoa into a Columbia watchglass. Several drops of 1% PTA, adjusted to pH 6.8 with 0.1 N NaOH, were added, and the sperm quickly dispersed in the fluid with a pipette. A drop of the suspension was then transferred to a Formvar-carbon-coated 200 mesh copper grid for from 2 to 10 minutes. Excess fluid was drained from the grid with filter paper and the preparation was allowed to dry. This material was examined with a Zeiss 9A electron microscope.

Each sample of spermatozoa was observed by phase contrast microscopy before negative staining, to insure that all spermatozoa used were healthy, as determined by their morphology and motility. In one experiment, spermatozoa from an animal in an advanced state of deterioration were used for comparison with the sperm of healthy animals.



FIGURES 1–3.

Observations

The mature spermatozoa of *Stylochus zebra* are approximately 35 μ long and 1.25 μ in diameter. A straight head is continuous with a corkscrew-shaped tail; a fine filament projects anteriorly (Fig. 1). There are no free flagella, but two lateral axial units with the "9 + 1" pattern (Henley, Costello, Thomas and Newton, 1969) adhere to the tail, lying in two grooves (or compartments) just beneath the cell membrane. A single row of from 100 to 120 cortical singlet microtubules is arranged just beneath the plasma membrane of the spermatozoon. In negatively stained preparations, the different microtubular elements (cortical singlets, axonemal doublets, and the complex core of the axial unit) can be identified readily (Fig. 2). The present account will be limited to a consideration of the cortical singlets and axonemal doublets of the spermatozoa.

Cortical singlets

Following negative staining, the cortical singlets usually fall on the grid in parallel array, either in straight or smoothly curved rows. Sharp bends, when they occur, are accompanied by clean breaks in the tubules (Fig. 3). The most obvious feature of the singlets is the arrangement of the subunits to produce a striking helical configuration, the pitch of which varies from 12° to 24°. The angles observed most frequently are between 16° and 19°. The alternating electron-dense and electron-lucent bands which make up the helix are repeated at intervals of *ca.* 80 Å. The diameter of these helical tubules is 200 to 240 Å. The pattern described may be interrupted in two ways:

(1) The helical pattern may become altered in spermatozoa from deteriorating animals (Fig. 4). The tubule tends to maintain its integrity rather than to collapse. However, it becomes spread laterally to a diameter of *ca.* 290 Å and stretched longitudinally so that the distances between the gyres of the helices are increased by *ca.* 15%. The subunits become visible, and lateral and longitudinal linkages between these subunits can be seen in places. Five longitudinal protofibrils can be counted under these conditions. With further spreading complete collapse of the tubule is often observed; the helical structure is lost entirely and 7 or 8 protofibrils can be seen in a flat plane.

(2) The helical configuration may also be interrupted, even in apparently healthy spermatozoa, when large regions of several adjacent microtubules undergo a transition to a ribbon-like structure, usually 350 Å in diameter (Figs. 5 and 6). At high magnification, these regions are seen to be composed of 6 or 7 protofibrils made up of subunits which give the fibrils a beaded appearance (Fig. 7). The actual size of the subunits cannot be measured with accuracy in negatively stained material, but their center-to-center spacing, measured along the length of the

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Figure 1. Mature spermatozoa of *Stylochus zebra* as observed by phase contrast microscopy. *h* = head. Magnification: 1600×.

Figure 2. Isolated microtubular complex of PTA-treated spermatozoon. Present are two 9 + 1 axonemes plus all the cortical singlets. *h* = head; *au* = axial unit; *c* = core of axial unit; *cs* = cortical singlet microtubules. Magnification: 7000×.

Figure 3. Cortical singlet microtubules with helical configuration revealed by negative staining. A break associated with a sharp bend is indicated by *V*. Magnification: 152,000×.

FIGURES 4–5.

protofibril, is *ca.* 40 to 45 Å. The lateral spacing of the protofibrils from center to center is *ca.* 50 Å, although at maximum spreading the distance may be much greater. These protofibrillar regions may continue as such until the microtubule ends distally, or may undergo a transition back to the helical form.

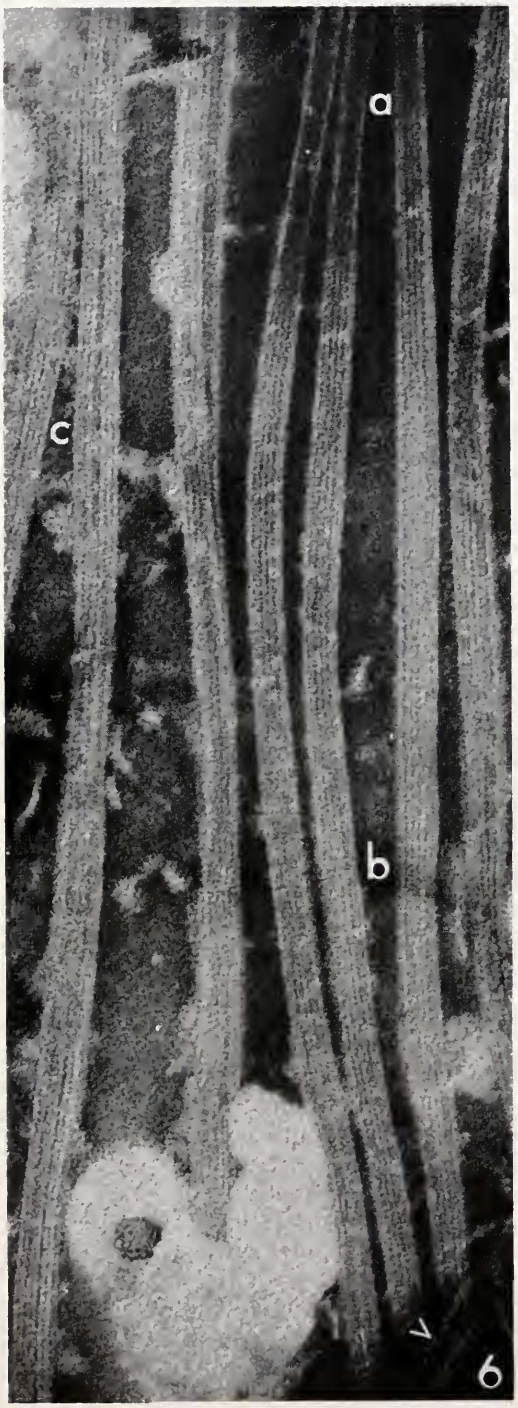
Axonemal doublets

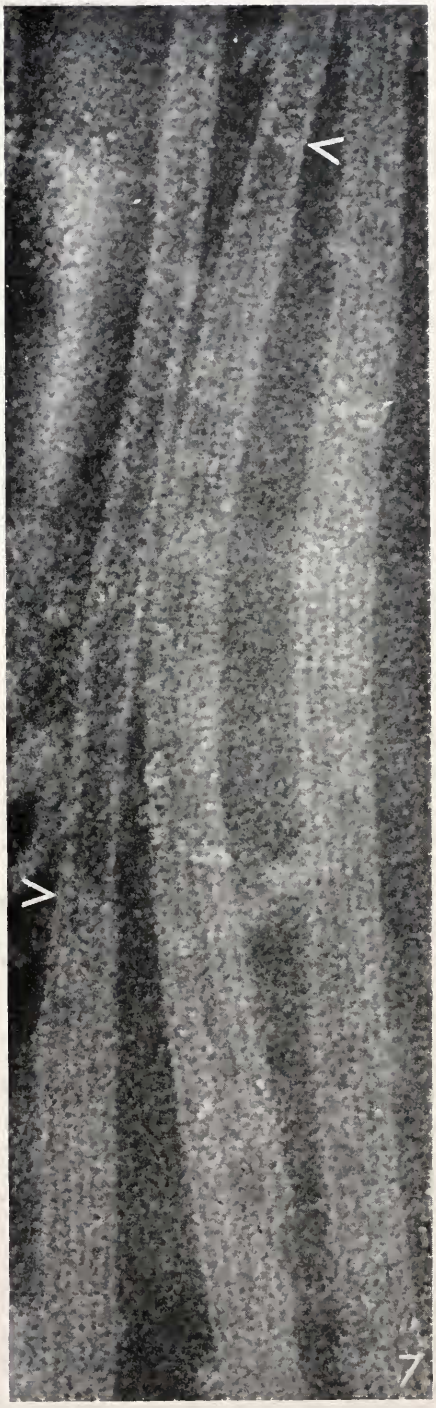
The 9 doublets of each axial unit, negatively stained, are approximately 440 to 480 Å in diameter in the relatively unmacerated state, although with increased maceration by PTA the diameter may be greater than 600 Å, especially in microtubules of degenerating spermatozoa (Fig. 11). Intact, negatively stained doublets show electron-lucent boundaries laterally and the two subtubules are separated by a median electron-lucent area *ca.* 80 Å wide (Fig. 8). In this material, even in tubules that have been macerated for only a short time, protofibrils can usually be seen. The protofibrils are made up of subunits which are spaced *ca.* 40 to 45 Å center-to-center along their lengths (Figs. 9 and 10). The lateral spacing of the protofibrils is relatively constant at *ca.* 50 Å, although in localized regions they may become more widely separated. With increased maceration, the electron-lucent median area, interpreted as the central partition separating the two subtubules, appears to split longitudinally into two protofibrils which often show cross-connections between them (Fig. 9). These protofibrils remain more electron-lucent than the other protofibrils, suggesting that they may represent two or more protofibrils superimposed on one another. The same is true for the protofibrils that occur laterally in each subtubule (Figs. 8 and 9). Occasionally one subtubule is completely lost, in which case the double set of protofibrils of the median wall usually remains (Fig. 9). Short regions of the doublet may also be seen to be turned on edge, in which case the diameter is the same as that of a single subtubule (Fig. 8).

In most cases in the PTA-macerated material, both subtubules of the doublet end distally at the same level. Occasionally, however, one subtubule drops out and the other continues as a single unit for as much as 2.5 μ, although the length of the remaining subtubule is usually only 1.5 to 2.0 μ (Fig. 14). Figure 13 shows that the disappearance of the shorter tubule results from its termination, rather than from rotation of the doublet. The electron-lucent protofibrils, interpreted to be the wall between the doublets, continue as part of the remaining tubule, maintaining their recognizable appearance for a short distance (Figs. 12, 13, and 14). The diameter of the remaining tubule is that of one subtubule including the common wall. At a distance of from 400 to 850 mμ from the point at which the first tubule drops out, the configuration of the remaining tubule changes abruptly into a helical pattern which is maintained for distances of up to 900 mμ. Regardless of the variation in the diameter of the tubule proximal to the helical portion, due to spreading, the diameter of the helix is from 250 to 260 Å. The center-to-center spacing between gyres is 80 Å and the pitch is usually 18° to 20°, but may vary

FIGURE 4. Cortical singlet microtubules of negatively stained spermatozoa from deteriorating animal. The microtubules are spread both laterally and longitudinally, and only traces of the original helical organization remain. Magnification: 152,400 ×.

FIGURE 5. Helical and protofibrillar configuration in cortical singlets of spermatozoa from healthy animals. Transitions are noted by *V*'s. Seven protofibrils can be counted in some regions, although six appear more frequently. Magnification: 152,000 ×.





FIGURES 6–7.

from 15° to 21° (Fig. 15). In a few instances the helix is interrupted for a short distance, beyond which the direction of the helix is reversed. This reversal is attributed to staining of opposite sides of the helix, rather than to an actual reversal in the direction of coiling.

The helical configuration usually does not continue to the end of the tubule; rather, the protofibrillar arrangement is observed at the terminus (Figs. 14 and 15). Six protofibrils can sometimes be counted at the extreme distal end.

A summary of the properties of the doublet and singlet microtubules is given below:

	Cortical singlet	Doublet subtubule
Diameter of helical tubule	200–240 Å	250–260 Å
Diameter of filamentous tubule	350 Å	260–400 Å
Gyres of helix (center-to-center)	80 Å	80 Å
Pitch of helix	16–19°	18–20°
Subunit spacing in protofibrils:		
Longitudinally (center-to-center)	40–45 Å	40–45 Å
Laterally (center-to-center)	50 Å or more	50 Å or more
Number of protofibrils/tubule	6–7	5–6

Discussion

Both the cortical singlets and at least one subtubule of axonemal doublets of the spermatozoa of *Stylochus* can assume a helical or protofibrillar configuration under certain conditions of maceration and negative staining with PTA. So far, in our experience, the doublet subtubule has been seen to acquire the helical pattern only at some distance from the point at which the other member of the doublet drops out. Other reports of the occurrence in doublet microtubules of the helical structure described are those by Pease (1963), André and Thiéry (1963) and Porter (1966). Pease showed a helical structure in subtubules that had been completely separated from one another. André and Thiéry demonstrated the helical pattern in one member of the doublet only, the A-tubule according to their interpretation. A micrograph by Silveira (Porter, 1966) of doublets from the spermatozoa of *Dugesia* shows a helical structure for one, or perhaps both, of the members of the doublet. The suggestion from these studies and from the observations reported here is that the subunits of at least one doublet subtubule tend in PTA to acquire a helical configuration similar to that of the cortical singlets of the spermatozoa of the lungfluke (Burton, 1966a, 1966b, 1968) and of *Stylochus*. This may indicate that basically similar subunits and bonds are involved in both classes of microtubules.

A comparison of the helical structure of the doublet subtubule with that of the cortical singlet reveals that within the limitations of error in measurement, the two

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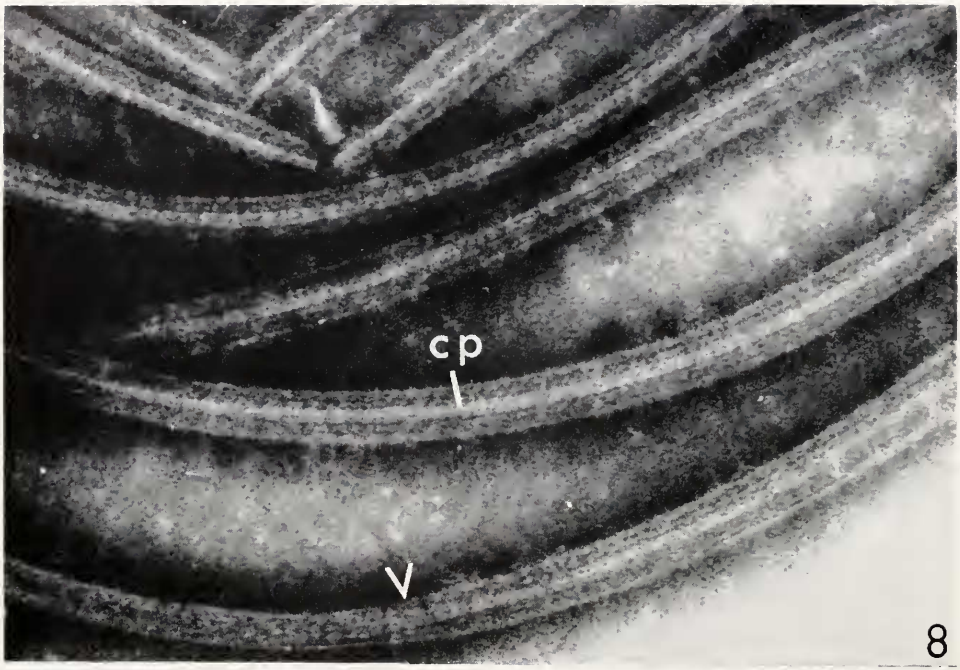
Figure 6. Cortical singlet microtubules showing varying degrees of separation of protofibrils. A region of transition from the helical to the protofibrillar condition is indicated by the *V* at the lower right. Tubules at *a* appear intact, but show no evidence of the helical pattern. Four or 5 protofibrils can be seen. With further separation, as in region *b*, 6 or 7 protofibrils can be counted. Equidistant lateral spacing of these protofibrils suggests the presence of lateral bonding. At *c* the protofibrils have completely separated in some regions. Magnification: 152,400 ×.

Figure 7. Transitions between helical and protofibrillar configurations in cortical singlets. The points at which the transitions occur are indicated by *V*'s. Magnification: 304,800 ×.



FIGURES 8–11.

types of microtubules differ in gross structure only in the diameters of the helices. If the differences in diameter would allow for the presence of 8 subunits per gyre in the cortical singlets (Burton, 1966a) and 12 or 13 subunits in the doublet subtubule (Ledbetter and Porter, 1964; Philips, 1966; Ringo, 1967), the numbers of subunits reported to compose each class of microtubules, then the contention that both classes of tubules are composed of equivalent subunits would be considerably strengthened. However, using the measurements presented here, the maximum number of subunits by which the two classes of tubules can differ appears to be two, which is two or three fewer than the number required. It is necessary to conclude, therefore, that either (1) the number of subunits in one or both classes of microtubules in spermatozoa of *Stylochus* differs from that reported for other organisms, or (2) the subunits are not equivalent in the two classes of microtubules. At this time it is not possible to decide between the two alternatives.

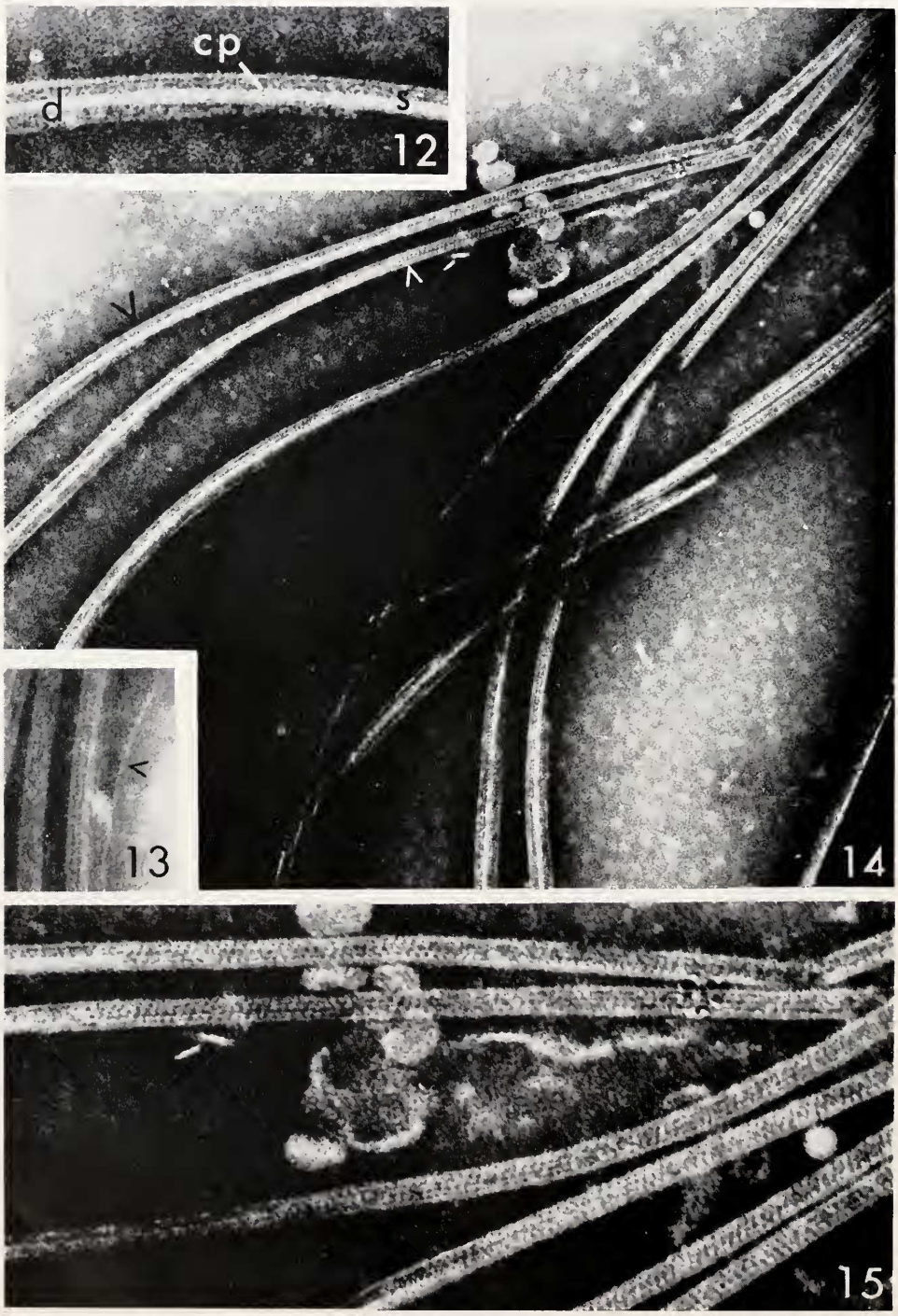
Examination of the fate of the common wall of the doublet at the distal end of the axial unit in negatively stained material may give some information about the relationship of that wall to each of the two subtubules. It has not been shown conclusively whether the wall belongs wholly to one subtubule, or whether it is equally shared by both members. Ringo (1967) has interpreted the wall as being common to both subtubules in the flagella of *Chlamydomonas*. In the sectioned material of Phillips (1966) the wall separating the two tubules of the axial unit of spermatozoa of *Sciara* is thickened; the author suggests that there may be some interpenetration of the walls of the two members of the doublet. In the same paper, however, he shows that in the spermatozoa of *Hippodamia* and *Draculacephala* the two subtubules of the doublet separate at the peripheral end; in sectioned material it can be seen that the A-subtubule is circular in cross-section, while the B-subtubule is C-shaped. Grimstone and Klug (1966) discuss the relationship of the wall to the two tubules in negatively stained material. They show that in relatively unmacerated doublets the common wall appears as a single line. Increased maceration results in a widening of the area and the appearance of two lines. Grimstone and Klug suggest that when the two lines appear, the subtubules have essentially separated from one another, and point out that when this occurs, both members of the doublet appear to be intact. Yet they state that after separation one subtubule collapses more readily than the other, indicating that the wall of one tubule may be incomplete. Since Grimstone and Klug suggest that upon separation of the doublet one member of the pair of "white lines" was contained in each subtubule, producing two presumably intact tubules, it is interesting that in *Stylochus* when one subtubule drops out, either along the length of the doublet or at the distal end, both lines representing the common wall continue along the

FIGURE 8. Doublet microtubules showing protofibrils and the electron-lucent central partition. The *V* marks a region in which one of the doublets is turned on edge. *cp* = central partition. Magnification: 152,400 ×.

FIGURE 9. Doublet microtubules showing 10 or 11 protofibrils. At *V* one subtubule has been digested away; the central partition maintains an association with the remaining subtubule. Note the cross-connections between the fibrils of the central partition. Magnification: 152,400 ×

FIGURE 10. Subunits of protofibrils of doublet microtubule. Magnification: 304,800 ×.

FIGURE 11. Two doublet microtubules of a spermatozoon from a deteriorating animal. Magnification: 152,400 ×.



FIGURES 12–15.

remaining subtubule. This may suggest that even in the intact doublets the common wall is more closely associated with one subtubule than the other.

It is not certain if the transformation at the distal end from doublet to singlet by loss of one member of the pair occurs naturally or results from the maceration by PTA. Since maceration by PTA has been observed to proceed from the distal to the proximal end of the axonemal complex (Costello, Henley and Ault, 1969), what is observed to be the termination of the doublets may be some distance proximal to the actual end of the doublets, due to the action of the PTA. If this is the case, and if one tubule is more sensitive to maceration (Grimstone and Klug, 1966), then the difference in the lengths of the two tubules may result entirely from the treatment. On the other hand, Satir has observed that in cilia of the mussel, the A-subtubule is consistently longer than the B-subtubule (Satir, 1967, 1968). Therefore, the difference in length reported here could reflect the situation in the normal axoneme. Some credence is given to this possibility by the fact that when one subtubule does remain, all 9 doublets show the phenomenon.

The significance of the existence of protofibrillar and helical forms in negatively stained material to the normal function of the microtubules is not apparent. Neither the helical nor the protofibrillar arrangement seems to be characteristic of the intact doublet, since negatively stained, unmacerated doublets rarely show either pattern, and helical configurations comparable to those shown here have never been demonstrated in sectioned material. The arrangement of the subunits may be far more complex, especially in the light of the 40, 80, 160 and 480 Å longitudinal periodicities described by Grimstone and Klug (1966) and others. The cortical singlets, however, appear to exist in the helical form in the untreated condition, since helices have been demonstrated in sectioned material (Burton, 1966a) and since they are present even in the earliest stages of PTA maceration. The natural occurrence of transitions to the protofibrillar condition is much more doubtful, however, since its occurrence is relatively infrequent and its location along the microtubules is unpredictable. In addition, it can be related to the extent of the PTA treatment.

It should be mentioned, however, that the transition from the helical to the filamentous condition described here is observed frequently enough to suggest that it is the result of the treatment, rather than of natural disintegration processes in

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FIGURE 12. Doublet microtubules near distal end of axial unit at the point at which one subtubule terminates. Note that the electron-lucent central partition continues along the remaining subtubule. This figure is an enlargement of the region near the black *V* in Figure 14. *d* = doublet microtubule; *s* = subtubule of doublet; *cp* = central partition. Magnification: 152,000 ×.

FIGURE 13. Portion of doublet microtubule at the point at which one subtubule disappears. The position and obvious termination of the protofibril denoted by the *V* show that the disappearance of the subtubule results from its termination, rather than from rotation of the doublet. Note that the central partition remains associated with the continuing subtubule. Magnification: 152,000 ×

FIGURE 14. Region near the distal end of an axial unit, showing the disappearance of one subtubule (black *V*) and the transition to the helical configuration in the remaining subtubule (white *V*). Note that in the subtubule denoted by the white *V* at least 4 protofibrils can be seen at the terminus. Magnification: 76,000 ×.

FIGURE 15. Enlargement of portion of Figure 14, showing the transitions to the helical configuration in one subtubule of several doublets. Four or 5 protofibrils can be seen at the terminus. Magnification: 152,000 ×

the spermatozoon, as described by Burton (1966b). Since the spermatozoa were removed directly from the vas deferens, the possible presence of degenerating spermatozoa from the seminal receptacle was virtually eliminated. In the experiment in which spermatozoa from deteriorating animals were negatively stained, transitions from helices to protofibrils were observed to occur more frequently; however, both doublet and singlet microtubules of degenerating spermatozoa have a characteristic "fuzzy" appearance, and the gyres of the helical singlets are spread apart and are irregular in outline. The transition to the protofibrillar arrangement can be seen in spermatozoa which show none of the irregularities characteristic of the degenerating spermatozoa.

I wish to thank Dr. Donald P. Costello and Dr. Catherine Henley for their suggestion and direction of the problem. Their constructive criticism and constant encouragement are gratefully acknowledged. I would also like to thank Miss Nancy C. Edwards for her help in the preparation of the typescript.

Summary

1. In spermatozoa of *Stylochus zebra* negatively stained with phosphotungstic acid, both cortical singlet and axonemal doublet microtubules can exist in a protofibrillar or a helical configuration. In cortical singlets the helical configuration appears more frequently; transitions to the protofibrillar condition occur at random and appear to result from the treatment with PTA. In doublet microtubules, the protofibrillar arrangement is the usual one. The helical structure is encountered only at the distal end of the axial unit in the single subtubule that persists distal to the termination of the other member of the doublet.

2. Comparison of the dimensions of protofibrillar and helical configurations in both singlets and doublets reveals that the two types of microtubules are similar. The observation that each type of microtubule can assume the characteristic configuration of the other type suggests that the structural differences of the two classes of microtubules result from alternative associations of equivalent subunits.

3. The central partition between the two subtubules of a doublet appears to be more closely associated with one of the two subtubules, since it remains when one subtubule drops out, either along the length of the doublet or at its distal end.

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